

HIV infection and hypertension

Epidemiological and pathogenetic aspects

PhD thesis

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and for bringing so much joy into my life.

ABBREVIATIONS

ABP	ambulatory blood pressure
AIDS	acquired immune deficiency syndrome
ART	antiretroviral therapy
BP	blood pressure
CHD	coronary heart disease
CI	confidence interval
CVD	cardiovascular disease
DAD	Data Collection on Adverse Effects of Anti-HIV Drugs
DBP	diastolic blood pressure
eGFR	estimated glomerular filtration rate
ESC	European Society of Cardiology
ESH	European Society of Hypertension
HDL	high-density lipoprotein cholesterol
HIV	human immunodeficiency virus
HT	hypertension
IQR	interquartile range
LPS	lipopolysaccharide
MD	myeloid differentiation
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
OR	odds ratio
PI	protease inhibitor
SBP	systolic blood pressure
sCD14	soluble CD14
SCORE	systematic coronary risk evaluation
SD	standard deviation
SMART	Strategies for Management of Antiretroviral Therapy
START	Strategic Timing of Antiretroviral Treatment
TLR	toll like receptor

LIST OF PAPERS

Paper I Hypertension and antihypertensive treatment in HIV-infected individuals. A Longitudinal cohort study

Ingjerd W. Manner, Morten Baekken, Olav Oektedalen, Ingrid Os

Blood Pressure, 2012;21:311-19

Paper II Low Nadir CD4 Cell Count Predicts Sustained Hypertension in HIV-Infected Individuals

Ingjerd W. Manner, Marius Trøseid, Olav Oektedalen, Morten Baekken, Ingrid Os

The Journal of Clinical Hypertension, 2013;15:101-106

Paper III Markers of microbial translocation predict hypertension in HIV-infected individuals

Ingjerd W. Manner, Morten Baekken, Dag Kvale, Olav Oektedalen, Maria Pedersen, Susanne Dam Nielsen, Piotr Nowak, Ingrid Os, Marius Trøseid

HIV Medicine, 2013; Epub Jan17

1 INTRODUCTION

1.1 Human immunodeficiency virus infection

1.1.1 History and epidemiology

A new syndrome of immunodeficiency was first described in San Francisco in 1981 and was later designated acquired immune deficiency syndrome (AIDS). The human immunodeficiency virus (HIV) was first isolated in France in 1983 [1], and the virus was soon extensively characterized (Fig. 1).

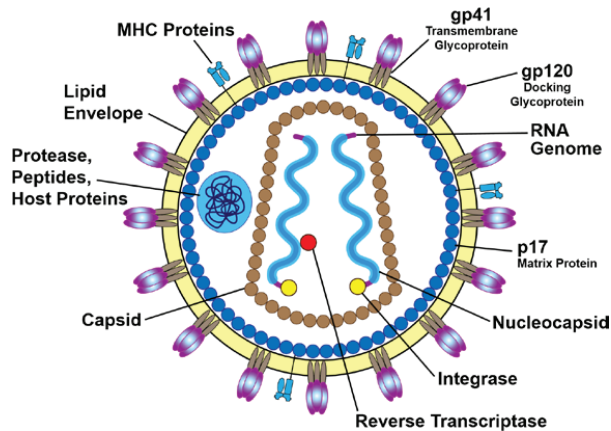


Figure 1. The human immunodeficiency virus (Wikipedia 2012).

The HIV epidemic evolved from primarily affecting men who have sex with men in the 1980's to a worldwide pandemic encompassing diverse populations. In 2011, around 34 million people were living with HIV (Fig. 2) [2]. The annual incidence reached its peak in 1997 when around 3 million people became newly infected with HIV (Fig. 2). Combined antiretroviral therapy (ART) was introduced in 1996, and access to ART has been massively scaled up during the last years. The mortality of AIDS has decreased, and the prevalence of HIV-infected persons is now slowly rising (Fig. 2) [2].

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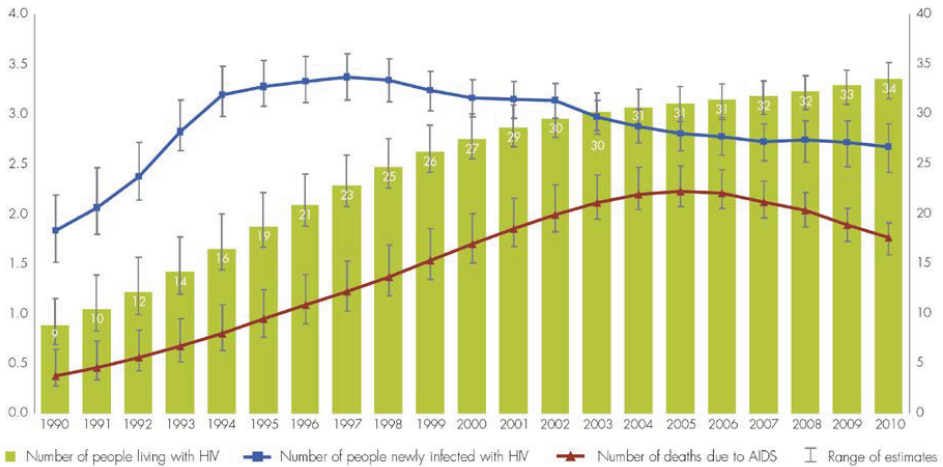


Figure 2. Number (millions) of people living with HIV, number of people newly infected with HIV and number of AIDS deaths worldwide, 1990–2010 (UN Millennium Development Goals Report 2012 [3]).

Despite increased access to ART, the global treatment coverage is still around 50%, with around 8 of 15 million eligible HIV-infected persons receiving ART [2]. Effective ART reduces the likelihood of HIV transmission [4], and increased availability of ART may contribute to reduce the incidence of HIV.

HIV infection is by far most widespread in Sub-Saharan Africa where 23 million people are living with HIV [2]. The dominating mode of transmission differs by geographic location. As conditions for the HIV infected population worldwide are extremely diverse, the term “HIV-infected population” hereafter designates HIV-infected individuals in the developed world, with available ART and health care resources.

In Norway, a total of 4900 people have been diagnosed with HIV 1984–2011, and an estimate of 3500–4000 people are living with HIV [5]. HIV dominates among heterosexual immigrants infected before arrival to Norway and among men who have sex with men, each group comprising about one third of the HIV-infected individuals. The HIV incidence in Norway has been rather stable during the last five years with around 270 persons being diagnosed with HIV each year [5].

1.1.2 Natural course and antiretroviral therapy

During the acute infection, viral replication expands in the absence of an immune response. In parallel with increasing viremia, the majority of patients develop mononucleosis-like symptoms. As the immune response evolves, viral load decreases and reach a set-point in which the viral load is stabilized, and the chronic phase is reached (Fig. 3) [6]. During the chronic phase, the infection is clinically latent. Viral replication continues during latency, particularly in lymphatic tissue, and there is a progressive loss of CD4⁺ T lymphocytes. As CD4 cell count drop, the patient develops constitutional symptoms. Due to loss of cell-mediated immunity, the patients are susceptible to a variety of opportunistic infections and cancers with development of AIDS which eventually lead to death [6].

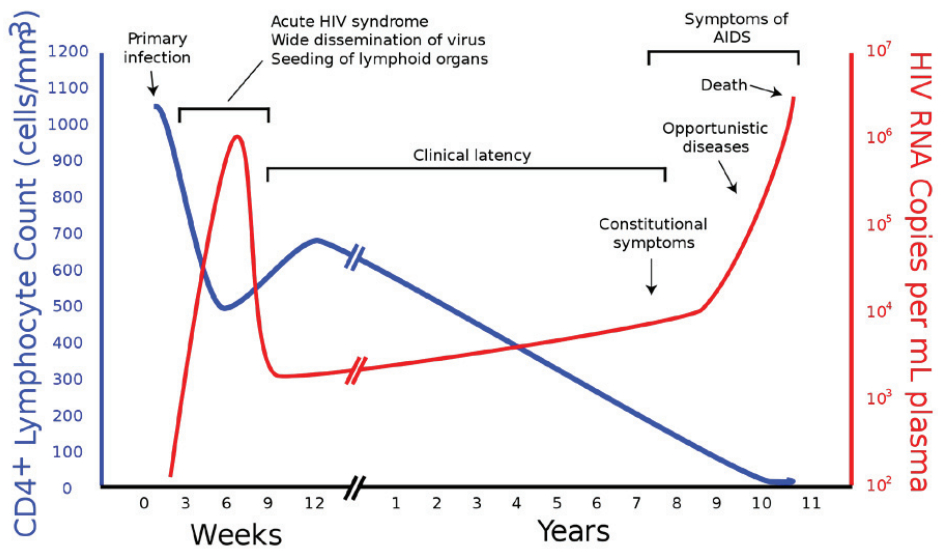


Figure 3. Graph of the relationship between viral load and CD4 cell count over the average course of untreated HIV infection (Wikipedia 2012).

Combined antiretroviral therapy includes a “backbone” of two different nucleoside reverse transcriptase inhibitors (NRTI) combined with a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). ART has changed HIV from being a fatal disease into a chronic, manageable infection with markedly reduced morbidity and mortality [2, 7]. Viral load and CD4 cell count are used to assess HIV

progression and the effect of ART. Successful treatment is defined by achievement of sustained viral suppression, i.e. persistent viral load < 50 copies/mL [8]. The optimal timing of ART initiation is a controversial key question. In asymptomatic patients, current European guidelines recommend initiation of ART at CD4 cell count below 350 cells/ μ L [8, 9], whereas recent federal US guidelines recommend initiation at CD4 cell count below 500 cells/ μ L [10]. The Strategic Timing of Antiretroviral Treatment (START) clinical trial; a large, ongoing randomized multinational study, will hopefully provide more definite answers to whether treatment should be started immediately or be deferred [11].

1.2 Non-AIDS morbidities in HIV

After the introduction of ART, increased longevity has been observed in people living with HIV. However, an increase in so called non-AIDS morbidities has been observed. Non-AIDS morbidities are defined as non-infectious diseases not directly caused by HIV or AIDS. The spectrum of non-AIDS morbidities includes non-AIDS-defining malignancies, liver, bone and renal diseases, cardiovascular diseases (CVD) including hypertension (HT), neurologic diseases and frailty, and often includes diseases related to increasing age [12]. An increased proportion of deaths in the HIV-infected population are caused by non-AIDS morbidities [13-16]. Non-AIDS morbidities seem to occur more frequently in the HIV-infected populations on ART than in the HIV-uninfected population [12, 17-19]. A premature ageing process has been suggested to occur in HIV-infected individuals [20], and altered immunocompetence seems to contribute to the increased burden of age-related non-AIDS morbidities [12, 13, 21-23]. Moreover, a number of additional factors may contribute to increased morbidity, e.g. risk behaviour, use of drugs and alcohol, life style, smoking and socioeconomic factors [12, 24]. Importantly, long-time cumulative exposure to ART does not seem to increase risk of non-AIDS related mortality [25].

1.2.1.1 Cardiovascular disease in HIV

Whether HIV infection in the era of ART contributes to increased cardiovascular risk is a subject to intense debate and also growing concern. HIV infection has been associated with indices of subclinical atherosclerosis, such as measures of endothelial dysfunction [26-29], increased arterial stiffness [30-32], increased carotid intima media thickness [33-35] and atherosclerotic progression [36]. Moreover, HIV-infected individuals have increased relative risk of CVD, including coronary heart disease (CHD) [37-39], acute myocardial infarction [39-45], stroke [45, 46], and peripheral vascular disease [47] compared to the HIV-uninfected population, even after adjustment for demographic and cardiovascular risk factors. The adjusted relative risk for myocardial infarction is reported to be around 1.5 times higher than that of the general population [39, 40].

Despite an increased relative risk of CVD in the HIV infected population, the absolute rates of CVD disease in the HIV-infected population are still low, and the incidence of CVD events [38, 45, 48] and CVD-related mortality [7] has in fact been reported stable or declining over the last years in spite of increasing age. However, in parallel with a globally ageing HIV cohort, an increased relative risk is likely to translate into increased absolute risk, and management of CVD risk will probably require increased attention from HIV physicians in the future.

1.3 Hypertension

1.3.1 Epidemiology and classification

Hypertension is defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg. Essential hypertension (i.e. hypertension without identifiable cause) constitutes around 95% of all cases of hypertension [49]. Both normotension (NT) and hypertension are graded into three classes (Table 1) according to the European Society of Hypertension (ESH)-European Society of Cardiology (ESC) guidelines for the management of arterial hypertension [50]. Known risk factors for hypertension include older age, male gender, black race,

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smoking, overweight, diabetes mellitus, dyslipidemia, sedentary life style and a family history of hypertension [50].

Table 1. Definitions and classification of blood pressure levels (mmHg) according to the ESH/ESC 2007 guidelines.

Category	Systolic Blood Pressure	Diastolic Blood Pressure	
Optimal	< 120	and	< 80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥ 180	and/or	≥ 110
Isolated systolic hypertension	≥ 180	and	< 90

The higher blood pressure should be applied if systolic and diastolic blood pressures fall into different categories.

Hypertension is a major risk factor for cardiovascular disease, and accounts for 17% of the total annual deaths in high-income countries [51]. There is a continuous linear relationship between blood pressure (BP) levels, even within normal range, and vascular mortality [52]. Hypertension often present in cluster with other conditions that increase risk of CVD [53]. Additionally, presence of multiple risk factors will potentiate cardiovascular risk [53].

The prevalence of hypertension rises steeply with increasing age [54]. The estimated prevalence of hypertension in high-income countries is 35% [55]. Overall, hypertension tends to be more prevalent in men [55]. In Norway, the Nord-Trøndelag health study (HUNT) reported an overall prevalence of 40% [56].

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Numerous cross-sectional studies have been conducted to establish the prevalence of hypertension in the general population. However, there is a paucity of prospective studies regarding incidence of hypertension in European populations [57-59], and data are not available with regard to incidence of hypertension in the Norwegian population.

1.3.2 Pathophysiology

The pathogenesis of essential hypertension is incompletely understood, and multiple genetic, environmental, metabolic, and demographic factors are at play and often interrelated. Abnormal renal salt handling in the kidney, intrinsic renal factors, the sympathetic nervous system, and renin-angiotensin system may all have important roles in the pathogenesis of elevated BP levels, with sustained vasoconstriction or volume overload [60]. With time, sustained BP elevation may induce structural and functional changes, leading to vascular remodelling and permanent increased peripheral resistance.

Hypertension and BP elevation have been related to inflammation with elevation of biomarkers of such as CRP, interleukin-6, and fibrinogen [61-63]. Moreover, increased prevalence of hypertension has been observed in inflammatory bowel disease, rheumatoid arthritis and periodontitis; diseases characterized by low-grade inflammation [64-67].

The immune system and immune cells may be both a cause and a consequence of hypertension. Hypertensive stimuli such as angiotensin II, high salt and increased sympathetic activity may induce production of cytokines and promote endothelial dysfunction and vascular inflammation [68-70] which in turn may stimulate further BP elevation through vasoconstriction [71].

1.3.3 Risk stratification

Several algorithms for CVD risk stratification have been developed, and the modified version of the original Framingham Risk Score of CHD is the oldest and

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most widely used [72, 73]. The model Systematic Coronary Risk Evaluation (SCORE) (Fig. 4) is developed for high and low-income regions of Europe [74].

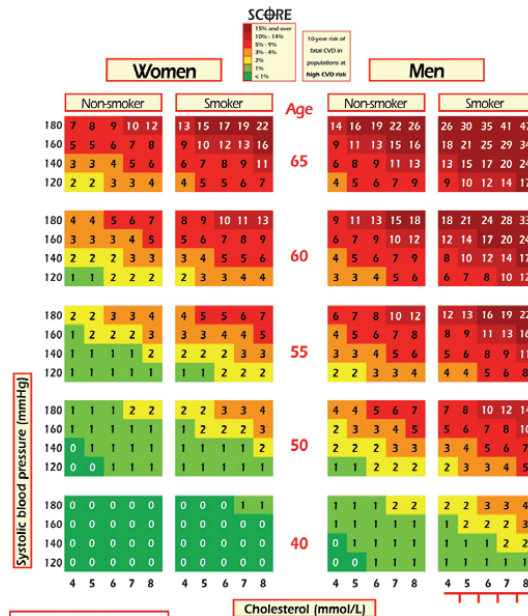


Figure 4. SCORE -European High Risk Chart of fatal CVD in high risk regions of Europe by age, gender, systolic blood pressure, total cholesterol and smoking status [74].

The SCORE model estimates 10 year risk of CVD-related mortality in the population based on age, gender, SBP, total cholesterol and smoking status. During the last years, effort has been made to develop CVD risk evaluation specific to HIV-infected individuals [75]. A HIV-specific 5 year risk equation of CHD (opposed to 10 year risk in Framingham) has been developed based on data from the D:A:D (Data Collection on Adverse Effects of Anti-HIV Drugs) study, incorporating data on specific antiretroviral drugs and cardiovascular risk parameters [76].

1.3.4 Hypertension in HIV-infected individuals

Our group has previously reported a 36% prevalence of hypertension in Caucasian HIV-infected individuals, comparable to that of an age-, gender - and BMI-adjusted

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Norwegian population [77]. The prevalence of hypertension in HIV-infected cohorts varies considerably, from 8 to 49% [77-84] and is reported both higher [79, 85], similar [17, 24, 77, 80, 86] and lower [23, 87] than in the HIV-uninfected population. Methodological issues and differences in patient populations are the most likely explanations of this discrepancy.

There are few data regarding incidence of hypertension in HIV-infected individuals. In the large, multinational D:A:D study from 2005, comprising around 9000 normotensive HIV-infected people mostly from Europe, the annual incidence of new-onset hypertension was reported around 6% [88].

Established risk factors for hypertension such as age, male gender, black race, increased body mass index, hyperlipidemia, and microalbuminuria are significant risk factors for hypertension also in the HIV-infected population [77, 79-81, 86, 89, 90]. As in the general population, both high-normal BP and hypertension are independent risk factors for myocardial infarction [44, 91, 92] and CVD events [14, 78] in the HIV-infected population. SBP has in fact been associated with greater relative risk of myocardial infarction in HIV-infected persons compared to matched HIV-uninfected controls [93]. Moreover, presence of hypertension seems to imply an increased probability of multimorbidity in HIV-infected individuals compared to uninfected control subjects [17].

We and others have reported the finding of a higher proportion of patients with non-dipping pattern (i.e. reduced nocturnal fall in systolic BP) in HIV-infected subjects with hypertension based on office BP compared to HIV-uninfected hypertensive subjects [94-96]. Different studies have reported prevalent hypertension in HIV-infected individuals to be related to HIV-specific factors such as lipodystrophy [79, 80, 97, 98] and higher CD4 cell count [23, 97]. Moreover, lower nadir CD4 cell count, i.e. the lowest CD4 cell count level in the individual history, has been related to elevation of BP after initiation of ART [99, 100]. Both prevalent hypertension and ambulatory hypertension (i.e. hypertension defined based on daytime ambulatory

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blood pressure (ABP)) has been independently related to duration of HIV infection [84, 101].

Several studies report ART-naïve HIV-infected patients to have lower BP levels than ART-exposed patients [81, 83, 102, 103]. Initiation of ART has been associated with development of hypertension and/or elevation of BP within the normal range in several studies [81, 99, 100, 104]. Cumulative exposure to ART-subclasses was not linked to elevated BP or development of hypertension in the D:A:D study on BP development [88]. However, cumulative exposure to ART has been independently related to prevalent hypertension in several [77, 81, 90, 105], but not all studies exploring this relationship [83, 86, 89].

The European AIDS Clinical Society (EACS) guidelines on non-infectious co-morbidities in HIV recommend that angiotensin-converting-enzyme inhibitors or angiotensin-II receptor blockers should be used as first-line drugs in HIV-infected non-black hypertensive individuals below 55 years, and as part of combined therapy in all patients [8]. As in the general population, the target BP level is <140/90 mmHg.

1.4 Established cardiovascular risk factors

As in the general population, established cardiovascular risk factors such as family history, age, male sex, hypertension, hyperlipidemia and smoking may contribute to CVD in the HIV-infected population [39, 40, 91, 106, 107]. The HIV population in Westernized countries is growing older, and by 2015, half the people living with HIV in USA will be older than 50 years [108]. Certain established risk factors are more prevalent in the HIV-infected population than in the general population, namely smoking [24, 82, 89, 92, 102, 109, 110] and presence of microalbuminuria [111, 112]. Use of ART has adverse influence on the lipid profile [24, 40, 90, 102, 113] and is associated with increased risk of diabetes mellitus [114-116] and CVD [19]. Unhealthy diet, low socioeconomic status, and excessive alcohol intake are likely more common in HIV-infected cohorts [23, 108] and may pre-date acquisition of HIV. Danish studies have reported mothers of HIV-infected patients to have

increased risk of myocardial infarction [117]. Moreover, HIV-infected smokers have excess morbidity and mortality rates compared to HIV-uninfected smokers [110, 118, 119]. The excess CVD risk among HIV-infected individuals may possibly have an influence on the BP levels and risk of hypertension.

1.5 HIV-specific factors

1.5.1 Immunodeficiency and immune restoration

Impaired immune restoration with permanent CD4 cell depletion in patients with suppressive ART is a predictor of both non-AIDS related morbidity [120, 121] and -mortality [121] as well as of all-cause mortality [15, 122, 123]. These observations are in line with the postulated accelerated ageing in HIV which could possibly contribute to the increased burden of age-related non-AIDS morbidities [12, 21]. However, in patients reaching CD4 cell count above 500 cells/ μ L, mortality rates are in fact comparable to the general population [124].

A history of progressive HIV with low nadir CD4 cell count has also been associated with later increased burden of non-AIDS morbidities [17, 125]. With regard to CVD, lower nadir CD4 cell count has been associated with endothelial dysfunction [126], increased arterial stiffness [127] and subclinical carotid atherosclerosis [34, 128, 129], but not with CVD events [78]. Furthermore, lower recent CD4 cell count has been associated with subclinical carotid atherosclerosis [129], arterial stiffness [30] and cardiovascular events [14, 38, 39, 78, 92] in studies including both patients with and without effective ART, but results are conflicting [22, 130].

No independent relation has been demonstrated between lower nadir CD4 cell count and prevalent hypertension [80, 84, 97, 131] or ambulatory hypertension [96, 101], although there has been an association in univariate analyses in some studies [84, 96]. The studies exploring this association have analysed nadir CD4 cell count as a continuous variable [80, 96, 101] or with a cut-off level at 100 or 200 cells/ μ L [84, 97, 131]. To the best of our knowledge, the association between previous advanced HIV infection and hypertension has not been studied.

1.5.2 Microbial translocation and chronic immune activation

Microbial translocation denotes the transport of bacterial products such as flagellin, bacterial DNA and lipopolysaccharide (LPS) from the gastrointestinal lumen through its epithelial layer into the systemic circulation. Microbial translocation occurs in a variety of conditions including chronic HIV infection [132, 133]. In early HIV infection, a massive selective loss of mucosal CD4 cells within the gastrointestinal tractus contributes to permanent enterocyte dysfunction and loss of mucosal integrity [134, 135]. In particular, there is a preferential loss of IL-17-producing CD4 cells [136] which is thought to contribute to increased permeability of the gut epithelium [137, 138]. LPS is a component of the gram-negative bacterial cell wall which can be measured in plasma, and LPS levels are commonly used to quantify microbial translocation [133, 138, 139].

LPS is a potent stimulator of the innate immune system, inducing cell activation and release of pro-inflammatory markers [140]. LPS stimulates macrophages, monocytes, and other cells of the innate immune system by interacting with a receptor complex consisting of Toll like receptor (TLR)4, myeloid differentiation (MD)-2 and CD14 [141], and CD14 is released in soluble form (sCD14) upon stimulation [142]. Elevated levels of sCD14 are often taken as indirect evidence of LPS stimulation [132, 142]. With suppressive ART, mucosal integrity is improved and microbial translocation is reduced, but not normalised [133, 143-147]. Thus, microbial translocation may occur in HIV-infected individuals at a permanent basis with consequential low-grade elevation of plasma LPS levels, i.e. low-grade endotoxemia. Microbial translocation is recognized as an important contributor to the persistent immune activation in HIV [133, 138, 144, 148-150].

Our finding of low nadir CD4 count as a predictor of hypertension prompted discussions about potentially involved mechanisms, and we chose to investigate the potential role of microbial translocation for several reasons. First, microbial translocation correlates with disease progression and CD4 cell depletion [151, 152], and elevated levels of sCD14 have been independently related to mortality in HIV

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[153]. Second, increased CVD risk has been linked to lack of CD4 restoration despite effective ART [38, 78, 121]. Incomplete immune recovery is in turn related to lower nadir CD4 cell count [121, 147, 154-157], persistent microbial translocation [144, 147, 148] and immune activation [157-159]. Moreover, microbial translocation has been related to endothelial dysfunction [160] and atherosclerosis in HIV-infected individuals [161].

Studies in the general population were also part of the rationale for investigating a potential link between microbial translocation. Low-grade endotoxemia has been connected to several conditions associated with hypertension including obesity, insulin resistance, and diabetes mellitus [162-166]; all conditions known to be associated with a state of chronic inflammation [167]. Furthermore, low-grade endotoxemia has been linked to development of atherosclerosis and risk of CVD [168-170], whereas CD14 has been associated with arterial stiffness in the general population [171]. However, microbial translocation as a potential trigger of hypertension has not been studied either in the HIV-uninfected or the HIV-infected population.

2 AIMS OF THE THESIS

The overall aims of the study were to describe hypertension in HIV-infected patients and to explore the possible relationship of HIV-specific factors and hypertension:

1. Describe the dynamics of hypertension status and assess the use of antihypertensive treatment and blood pressure control in hypertensive HIV-infected individuals (Paper I).
2. Assess the incidence and identify the predictors of new-onset hypertension in a longitudinal study (Paper I).
3. Explore the relationship between measures of HIV-induced immune dysfunction, microbial translocation and hypertension (Paper II and III).

3 METHODS

3.1 Ethics and concession

Oral and written information were given to the patients during a regular clinical visit, and written consent was required to participate in the study. The study was approved by the South-Eastern Regional Committee for Medical and Health Research Ethics, and concession was obtained from the National Data Inspectorate. Blood samples were collected and stored with consent from the patients and according to the regulation given for biobanks in Norway. The consent included possibility to retrieve blood samples collected prior to the current study from the local biobank for specified analyses.

3.2 Study design and participation

3.2.1 Paper I and II

In this epidemiological study, the design was that of a prospective cohort study in order to assess BP elevation and development of hypertension in an HIV-infected population.

All HIV patients attending the Outpatient Clinic at Department of Infectious Diseases at Oslo University Hospital, Ullevål were invited to participate in the baseline “Microalbuminuria in the HIV-infected population of Oslo” (MAHO) study in 2004–2005 [77, 112]. No exclusion criteria were applied. All patients completing that study were invited to participate in a follow-up study (Fig. 5). Of a total of 597 patients, 542 patients completed the MAHO study, and 434 patients (80%) attended the follow-up study which was conducted during the years 2007–2010.

METHODS

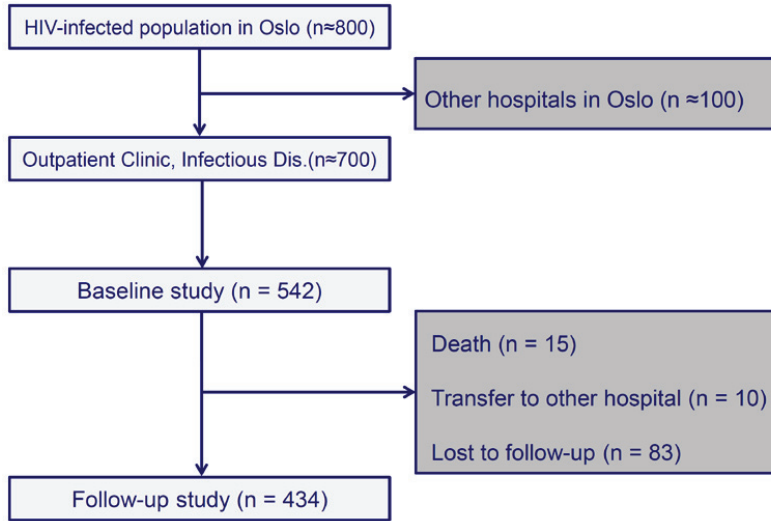


Figure 5. Flow chart of the HIV-infected cohort in the longitudinal study.

Eighty-three patients declined the invitation to participate in the follow-up study (Fig. 5). There was no difference between the 108 persons who did not attend the follow-up study compared to the 434 persons who completed the study with regard to distribution of risk groups, the proportion of Caucasians, smokers, patients with diabetes mellitus or hepatitis C, drug abusers or the proportion of ART-naïve patients. Furthermore, there was no difference with regard to BMI, eGFR, urinary albumin excretion, CD4 cell count, HIV RNA, duration of ART or HIV duration. Conversely, the individuals who did not attend the follow-up study had lower BP, were more often normotensive (80.6 vs. 65.2%, $p = 0.002$) and had lower cholesterol compared to those who completed the longitudinal study (Paper I).

Data from all the 434 persons, including 19 persons who did not attend all three visits were included in the final analyses. The follow-up study was completed after 39 (interquartile range (IQR) 35–45, range 20–69) months. The median time period between the first and the third visit during baseline and follow-up was 6.1 (IQR 3.4–18.1) and 5.5 (3.0–9.7) months, respectively ($p < 0.001$).

3.2.2 Paper III

In this substudy, data from a selected group of participants in the follow-up study were explored (Fig. 6). Blood samples had been collected before the inclusion in the MAHO study and were retrieved from the biobank for analysis of LPS and sCD14.

The following criteria were used for the recruitment to the substudy:

- Either sustained normotension or sustained hypertension both at baseline and at follow-up.
- Either high (i.e. > 200 cells/ μ L) or low (i.e. < 50 cells/ μ L) nadir CD4 cell count
- Availability of plasma samples at the time of nadir CD4 count
- Individuals with diabetes mellitus, known hypertension or exposure to ART before or at the time of nadir CD4 count were excluded.

Availability of plasma samples turned out to be a major limitation for recruitment. All eligible patients with available samples in the low nadir group (HT and NT) as well as in the high nadir HT group were included. Since matching across the two nadir strata was not possible, we chose to increase statistical strength by enlarging the high nadir NT group. Recruitment to this group was performed in a random fashion. A total of 42 participants could be included in the substudy (16 hypertensive (low nadir, $n = 10$) and 26 normotensive (low nadir, $n = 5$). A control group of 15 HIV negative Danish Caucasians were recruited by advertisement in local newspapers. Although no formal matching was performed, the age and gender distribution was similar to the HIV-infected study participants.

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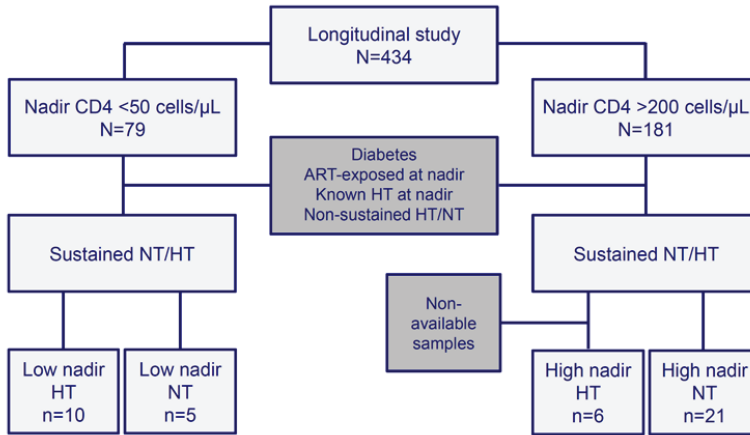


Figure 6. Flow chart of the recruitment of HIV patients to the substudy (paper III).
HT, hypertension; NT normotension; ART, antiretroviral therapy.

3.3 Data collection

3.3.1 General data

Data on smoking, CVD and diabetes mellitus were obtained from a questionnaire. Clinical, laboratory and demographic data (birth of date, body weight, gender, date of first HIV positive test, hepatitis status, ethnicity, risk group, death, CD4 cell count (nadir and present), HIV RNA and use of ART) were extracted from the local HIV database at the inclusion time at baseline, at follow-up (paper I and II), and at the time point of nadir CD4 count (paper III). Data on routine laboratory tests, antihypertensive treatment throughout the longitudinal study period and confirmation of the diagnosis of diabetes mellitus were also retrieved from hospital records. Data on BP at the time of nadir were explored in hospital records but such data were missing in the majority of the patients.

Glomerular filtration rate (GFR) was estimated according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation by Levey et al. $(\text{GFR} = 141 \times \min(\text{Scr}/\kappa, 1)^\alpha \times \max(\text{Scr}/\kappa, 1) - (1.209 \times 0.993 \times \text{Age} \times 1.018 [\text{if female}] \times 1.159 [\text{if black}]))$, where Scr is serum creatinine in mg/dL, κ is 0.7 for

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females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr / κ or 1, and max indicates the maximum of Scr / κ or 1) [172]. The CKD-EPI formula was used because it is assumed that GFR is estimated more accurately by this formula than by the Modification of Diet in Renal Disease (MDRD) formula, especially in the higher GFR range [172].

HIV duration was recorded as the duration since the first seropositive test at the time of inclusion at baseline, at follow-up, and at the time point of nadir CD4 count. Duration of ART was calculated as the cumulative exposure to a triple class regimen, and duration of PI use was calculated separately. Patients who had never been exposed to ART were defined as ART-naïve whereas current ART was defined as ongoing ART exposure at the different time points.

Nadir CD4 cell count was recorded as the lowest CD4 cell count ever before inclusion at baseline. Nadir CD4 cell count was recorded regardless of ART status at the time of nadir; 80% were ART-naïve whereas 11% of the patients were on current ART at this time point. HIV RNA was recorded close to the date of nadir when available (i.e. from 1996), as well as at inclusion at baseline and follow-up.

The 10 year risk of fatal cardiovascular disease was estimated with the European SCORE model for high-risk countries (Fig. 4) [74]. Hypertensive subjects were stratified into four risk groups based on grade of hypertension and the number of cardiovascular risk factors at follow-up [50].

3.3.2 Laboratory analyses

Blood samples were taken as per clinical routine, and fasting state was not required. Urine albumin and creatinine were measured using an immunoturbimetric method (antihuman serum albumin antibody from Roche, Basel, Switzerland) and an enzymatic method (Roche). HIV RNA in EDTA plasma was quantified using polymerase chain reaction amplification with a COBAS Amplicor HIV-1 Monitor Test (Roche Diagnostics, Branchburg, NJ, USA). CD4 cell count was determined

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by routine flow cytometry using TriTest CD4/CD8 with TruCount Tubes (Becton Dickinson Biosciences, San Jose, CA, USA).

3.3.3 Blood pressure measurements

Blood pressure was measured in duplicate at three consecutive study visits, days to months apart both during baseline and follow-up. Blood pressure was measured in a relaxed sitting position in a quiet room after 5 minutes of rest using a semiautomatic oscillometric device (Omron M4, Matsusaka Co. Ltd, Matsusaka, Japan) with appropriate cuff size.

3.3.4 Urine analyses

Urinary albumin-to-creatinine ratio (ACR), a measure for the urinary albumin excretion rate, was determined in a urine specimen at each visit. A first void morning urine was preferred, but random spot urine specimens were not discarded as the reflection of 24-hour urinary albumin excretion was considered acceptable [173]. Urine samples were sent and analysed promptly at the Department of Clinical Chemistry, Ullevål University Hospital. A urine dip-stick test was performed, and if pyuria was present, the sample was discarded for further analysis as interference with the urinary albumin level was assumed. The average of three ACR measurements was used in the statistical analyses.

3.3.5 Lipopolysaccharide and soluble CD14

At the Outpatient Clinic of Infectious Diseases, blood samples taken during clinical visit have routinely been frozen at -20°C. These biobank samples were retrieved for analyses of LPS and sCD14 in the substudy (paper III).

LPS was analysed by Limulus Amebocyte Lysate colorimetric assay (Lonza, Walkersville, MD, USA). Samples were diluted 10-fold to avoid interference with background colour, and preheated to 68°C for 12 minutes prior to analyses to dissolve immune complexes as previously described [143]. Test samples were mixed with the LAL in the test kit and incubated for 10 minutes at 37°C before a substrate solution was mixed with the LAL sample and incubated for additional six

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minutes. The reaction was then stopped with stop reagent. If endotoxin was present, a conversion to yellow developed, and the absorbance of the sample was determined spectrophotometrically and concentration calculated from a standard curve. Assays were done in duplicate.

Soluble CD14 was analysed according to the manufacturer's instructions (ELISA R&D, Minneapolis, MN, USA). Briefly, samples were diluted 3000-fold, and incubated in duplicates at room temperature for three hours. After a total of four washing steps, the wells were incubated for one hour with sCD14 conjugate solution. Next, the wells were washed, substrate solution was added, and the wells were incubated for another 30 minutes before the reaction was stopped with a stop reagent. A conversion to blue developed if sCD14 was present, the absorbance of the sample were determined spectrophotometrically and the concentration was calculated from a standard curve.

To minimize the inter- and intra-assay variability, samples from hypertensive and normotensive patients at the time of nadir CD4 count were analysed in the same run, and all samples were run in duplicates. The 15 controls were analysed separately but with the same batch and with similar value for the positive control.

3.4 Definitions

The average of six systolic and diastolic BPs was used for statistical analyses both at baseline and at follow-up. Hypertension was defined as an average SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or reported use of antihypertensive therapy. Mean BP was calculated as $DBP + 1/3 \times (SBP - DBP)$. New-onset hypertension was defined as normotension at baseline and hypertension at follow-up. Sustained hypertension was defined as present hypertension both at baseline and at follow-up. BP level was classified according to the ESH-ESC 2007 guidelines (Table 1) [50]. Adequate BP control in patients on antihypertensive treatment was defined as an average SBP < 140 mmHg and DBP < 90 mmHg based on BP measurements taken after initiation of antihypertensive therapy. Microalbuminuria was defined as ACR between 2.5 and 30 mg/mmol in two or three urine samples. Abnormal urinary

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albumin excretion was defined as the presence of microalbuminuria or ACR between 30 and 150 mg/mmol, i.e. low-grade proteinuria. Hyperlipidemia was defined as total cholesterol ≥ 6.2 mmol/l. Body mass index (BMI) was calculated as kg/m^2 . Overweight and obesity was defined as BMI ≥ 25 and 30 kg/m^2 , respectively.

3.5 Statistical analyses

SPSS software (PASW Statistics, versions 17.0–18.0 and IBM Statistics, version 19.0–20.0) was used for statistical analyses. Data were presented as means \pm standard deviation (SD), number or percentage, or if skewed, as median and 25th and 75th percentiles (IQR). Between-groups comparisons were performed using either t-test or the Mann-Whitney test as appropriate. Comparison of within-group data were performed using a paired t-test, McNemar's test, or Wilcoxon signed rank test depending on the distribution of data. Categorical data were compared using chi-square test, and linear trend were tested using chi-square linear-by-linear association. Correlation analyses were performed calculating Spearman's (ρ) or Pearson's (r) coefficients as appropriate. Several variables were additionally analysed as categorical variables based on clinical relevance; BMI and baseline CD4 cell count were dichotomized, whereas ART duration was divided into four groups. Nadir CD4 cell count was dichotomized with 50 cells/ μL as cut-off as this is a threshold for very advanced immunodeficiency [6, 174].

Three different multivariate logistic regression models were created. The first model examined independent predictors of new-onset hypertension (paper I), the second model explored determinants of persistent hypertension among those who were hypertensive at baseline (paper I), and the third model examined independent predictors of sustained hypertension in the whole study cohort (paper II). The odds ratio (OR) for outcome were calculated in each model. Additionally, multivariate linear regression models were created to determine independent predictors of BP level (SBP, DBP and mean BP) (paper III). Independent variables were selected based on significant associations between outcome in bivariate analysis ($p < 0.1$) or

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known clinical relevance. The number of independent variables was adjusted according to the number of subjects with outcome. Spearman's correlation test was used to check for collinearity, and HIV duration and duration of ART were consequently not entered into the same regression models in paper I and II ($\rho = 0.58$ and $\rho = 0.65$, respectively). As SBP and DBP were intercorrelated, mean BP was calculated and entered into the multivariate logistic regression models in paper I. A statistically significant level was set at $p < 0.05$.

4 SUMMARY OF RESULTS

4.1 Paper I

Hypertension and antihypertensive treatment in HIV-infected individuals. A longitudinal cohort study

In a longitudinal study of 434 HIV-infected individuals (43 ± 11 years, 72% males, 71% Caucasians), the prevalence of hypertension at 35% did not change during the follow-up time (3.4 ± 0.8 years). The incidence of new-onset hypertension was 29.8 per 1000 person-years (95% confidence interval (CI) 20.3–42.2). HIV duration, mean BP and abnormal urinary albumin excretion remained independent predictors of new-onset hypertension after multiple adjustments. Use of antihypertensive treatment increased threefold from 17 to 49% in hypertensive patients. Adequate blood pressure control was found in 22% of patients on antihypertensive therapy.

4.2 Paper II

Low nadir CD4 cell count predicts sustained hypertension in HIV-infected individuals

In a longitudinal study of blood pressure in an HIV cohort of 434 patients, both nadir CD4 cell count < 50 cells/ μL (adjusted OR, 2.48; 95% CI, 1.27–4.83) and increased duration of ART (adjusted OR, 1.13, 1.03–1.24) were independent predictors of sustained hypertension throughout the study period. Older age, male gender, BMI > 25 kg/m² and baseline CD4 cell count ≥ 200 cells/ μL were also independent predictors of sustained hypertension. The predictive power of ART duration was more pronounced in patients with nadir CD4 cell count < 50 cells/ μL compared to patients with nadir CD4 cell count > 200 cells/ μL (OR, 1.48; 95% CI, 1.19–1.83 and 1.14, 1.00–1.31, respectively).

4.3 Paper III

Markers of microbial translocation predict hypertension in HIV-infected individuals

In this exploratory study of 42 HIV-infected patients (median age 42 (IQR 32–46) years; 79% men, 81% Caucasians, 38% hypertensive), plasma levels of lipopolysaccharide (LPS) and soluble (s) CD14 were measured retrospectively at the time of nadir CD4 cell count. LPS levels correlated with sCD14 in the whole study population ($r = 0.48$; $p = 0.001$) as well as in the subgroup with hypertension (Spearman's $\rho = 0.62$; $p = 0.011$). Both LPS and sCD14 were correlated with nadir CD4 cell count ($r = -0.57$; $p < 0.001$ and $r = -0.66$; $p < 0.001$, respectively). There was a stepwise increase in the number of patients with hypertension across tertiles of LPS ($p = 0.001$) and sCD14 ($p = 0.007$). Both LPS and sCD14 independently predicted subsequent blood pressure levels after adjustment for age and gender. For each 10 pg/ml increase in LPS (range 66–272 pg/ml), the adjusted increment in SBP and DBP during the first period of blood pressure recording was 1.03 (95% CI 0.23–1.83) mmHg ($p = 0.013$) and 0.78 (0.29–1.26) mmHg ($p = 0.002$), respectively.

5 DISCUSSION

5.1 Methodological considerations

5.1.1 Study population and design

Of approximately 700 possible study participants, 77% ($n = 542$) completed the MAHO study, and 80% ($n = 434$) of those were included in the follow-up study (Fig. 5). This single center population was assumed to be representative of an urban HIV population in a developed country with free access to health care.

Demographic and clinical characteristics did not change during follow-up (paper I). However, normotension was less prevalent in the study population (65.2%) than in the source population (68.3%) [77]. The conclusions regarding the incidence of hypertension may therefore be an underestimate.

The follow-up period of the longitudinal study was rather short, and considerable changes were not to be expected. A longer follow-up period would probably have resulted in more cases with new-onset hypertension and increased the statistical power. Moreover, a control group would have strengthened the results (paper I). The confirmation of hypertension status throughout the study period (i.e. sustained hypertension) ensured proper classification, and the use of sustained hypertension as an outcome may therefore have reinforced the conclusions in paper II and III.

Due to intra-individual variability of BP, multiple measurements over time will better reflect the “true” BP level in the individual patient [50]. The interval between the study visits was usually three months; however, the intervals could vary substantially according to the clinical visits.

Putative mechanisms for the link between low nadir CD4 count and hypertension were explored (paper III). To ensure homogeneity, only ART-naïve patients were included. Moreover, we chose to include patients with previous severe immunodeficiency or preserved immunocompetence as expressed by nadir CD4 cell count. Matching across the two nadir strata was unfortunately not manageable. The

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HIV-related characteristics in the normotensive and hypertensive patients did not differ across the two nadir groups (data not shown), thus minimizing possible HIV-related confounding factors. Data on BP levels at the time of nadir CD4 count were available only in selected patients, and care was taken to exclude those with hypertensive BP readings. The lack of data on BP may be a limitation of the study as one cannot ensure homogeneity of normotensive status at the time of nadir. A prospective design would be ideal to study the relation between microbial translocation, BP elevation and development of hypertension.

5.1.2 Data collection

5.1.2.1 Clinical data and laboratory data

The local HIV database enabled detailed recording of clinical, demographic and important HIV-related characteristics. All laboratory analyses were highly standardized and performed at a single centre.

The study population was moderate in number compared to large-scale multicentre studies, and a large proportion of patients had been exposed to frequently changing ART regimes. Detailed recording of exposure to separate drugs or different drug classes other than PI was consequently not considered to be of purpose. Yet, cumulative exposure to ART was assumed to reflect increased risk of toxicity and adverse effects. With the insight of the SMART (Strategies for Management of Antiretroviral Therapy) study, which demonstrated harmful effects of intermittent ART [175], assessment of ART interruption could also have been of value.

Ideally, HIV duration should have been calculated from the time of transmission, and using the date of the first seropositive test as starting point have possibly underestimated the HIV duration. However, a high proportion in our cohort lacked data on a previous seronegative test, and other methods of calculation were inapplicable. The calculation of HIV duration in our study was in accordance with that of other studies on HIV.

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Nadir CD4 cell count was recorded as the lowest CD4 cell count ever, without taking into account concurrent interruption or use of ART. Consequently, patients would differ with regard to virologic status at the time of nadir. In the substudy (paper III), however, care was taken to include only patients who reached nadir CD4 cell count before initiation of ART.

Different studies have reported lipodystrophy to be independently associated with increased BP [176], development of hypertension [88], and prevalent hypertension [79, 80, 97, 98]. It is possible that our finding of an adverse impact of ART could be partly attributed to an effect of lipodystrophy. Hence, recording of change in body composition would possibly have added value to the study.

In the longitudinal study there was a lack of biomarkers related to HIV pathogenesis, such as biomarkers of inflammation and immune activation. This is considered as a major limitation of the longitudinal study (paper I and II).

5.1.2.2 Blood pressure measurements and classification of hypertension

Blood pressure measurements were performed in a standardized manner throughout the study and by the same experienced nurses, which ensured quality.

The probability of white coat hypertension decreases when the diagnosis of hypertension is based on repeated BP measurements over time [177]. Clinical guidelines on diagnosis of hypertension recommend at least two BP measurements performed at several separate occasions over a prolonged period of time [50, 178]. Classification of hypertension in our study was based on the use of antihypertensive therapy or the average of six BP recordings performed at three different visits. This method was selected to increase reliability of classification of hypertension status.

Blood pressure is subject to physiological variability, and use of more than two BP measurements per visit is recommended if the first two measurements differ substantially [50]. We measured BP only in duplicate during the study visits. Still, the SD of the differences between the duplicate SBP/DBP measurements at each visit was 7.9–9.3/5.1–6.6, indicating high reproducibility [179].

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White coat hypertension can only be assessed using ABP [180]. ABP measurement and self-monitoring of BP over a period of time are useful methods to confirm hypertension [50, 177, 181]. ABP was monitored in 77 hypertensive patients (based on office BP) in the MAHO study, and white coat hypertension was present in 26% [101]. The prevalence of white coat hypertension in the general population is estimated to be around 20% [180].

Large epidemiological studies are often based on one single BP measurement and may therefore overestimate the presence of hypertension. Additionally, comparison of studies on hypertension is challenged by differences in the number of BP measurements, the length of the study periods, the number of study visits, as well as the methods of BP measurement. The use of 24-hour ABP measurement would have strengthened our study.

5.1.2.3 Evaluation of cardiovascular risk

Framingham Risk Score or the D:A:D risk equation were not used in the present study due to missing data on HDL-cholesterol in a substantial proportion of patients. Cardiovascular risk was therefore calculated using the SCORE model, recommended by the Forth Joint European Task Force [74]. The SCORE model has been compared with the Framingham 10 year Risk Score of CHD in a Spanish HIV-infected cohort, with agreement in 83% [182].

5.1.3 Statistical methods

BMI as well as baseline and nadir CD4 cell count were entered as dichotomized variables into the different multivariate logistic regression models (paper I and II). This may have reduced the statistical power, but increased the clinical relevance. In paper III, markers of microbial translocation were independent predictors when creating a linear regression model with BP as outcome, strengthening the reliability of the association with hypertension.

A small sample size will affect statistical power and increase the risk of overlooking true associations, i.e. type II errors. On the other hand, the risk of type I errors with

finding of untrue associations will be less probable. Thus, the significant independent predictors of hypertension demonstrated in the present study are likely to be reliable.

5.2 Discussion of the results

5.2.1 Prevalence of hypertension

The prevalence of hypertension remained unchanged during the follow-up period. Considerable dynamics were observed in the hypertension status; however, the number of patients with new-onset hypertension at follow-up was identical to the number of hypertensive patients converting to normotension (Paper I). During follow-up, eGFR decreased and the proportion with diabetes mellitus increased. That could affect BP level in the population. Moreover, the estimated CVD risk was higher at follow-up (paper I). Many patients had their BP recorded for the first time in this study; this may have induced life style changes with lowering effect on BP. The short period of follow-up and the low age of the patients (majority < 50 years) may explain the unchanged prevalence of hypertension.

5.2.2 Incidence of new-onset hypertension

In paper I, we assessed the incidence of new-onset hypertension. Blood pressure levels in the general population differ across countries [54, 183], and comparison with Norwegian data on incidence of new-onset hypertension would be desirable. Unfortunately, no published data are available on new-onset hypertension in the general population in Norway. In studies of middle-aged general populations in Western Europe [57-59] and of Caucasians in USA [184, 185], the reported annual incidence (2.4–4%) was comparable to that of our study, namely 3.1% (paper I). However, different methods of BP assessment as well as differences in geographic location, age, gender, BMI, and BP levels at inclusion make comparison challenging.

There is a scarcity of data regarding incidence of hypertension in people living with HIV. An incidence in the range of 90–430/1000 person years was reported in a

selected cohort of older HIV-infected individuals [186]. In the D:A:D study from 2005, the annual incidence of new-onset hypertension in HIV-infected persons was reported to be 72 per 1000 person-years, corresponding to an incidence of around 6% per year [88]; around twofold of the incidence of new-onset hypertension found in our study (3.1% or 29.8 per 1000 person-years) as well as in the general population in Europe [57-59]. Interestingly, another D:A:D paper from the same time period reported the prevalence of hypertension to be 23% [91], probably comparable to that of the middle-aged population in Europe [54]. Significant risk factors for hypertension in the D:A:D study (age, BMI, proportion with high-normal BP at baseline) were comparable to those in our study. Nonetheless, differences in the distribution of risk factors for hypertension, size and origin of study population, criteria for the classification of new-onset hypertension and methods of BP measurements may have been the explanation of the disparate findings compared to our study.

5.2.3 Impact of traditional risk factors on hypertension

We examined whether traditional risk factors for hypertension in the general population would be independent predictors of new-onset hypertension (paper I), sustained hypertension (paper II), and later BP level (paper III) in a HIV-infected cohort. In paper I, baseline BP and abnormal urinary albumin excretion were the only independent predictors of new-onset hypertension. The limited number of participants included in the multivariate analysis in paper I ($n = 283$) and paper III ($n = 42$) may have caused insufficient statistical power to demonstrate impact of other relevant traditional risk factors. In paper II, individuals with sustained HT had higher prevalence of several traditional risk factors compared to the other study participants, and older age, male gender, and overweight were all independent predictors of sustained hypertension. The fact that these well-known traditional risk factors for hypertension turned out as independent predictors in our small-scale study was reassuring and strengthened the possibilities of finding true HIV-related predictors of hypertension.

5.2.4 Impact of HIV-specific factors on hypertension

5.2.4.1 *Current and nadir CD4 cell count*

In paper II, we found that both previous low nadir CD4 cell count (i.e. nadir CD4 cell count < 50 cells/ μ L) and current CD4 cell count > 200 cells/ μ L were independently associated with sustained hypertension after multiple adjustments. Others have also reported an independent relation between present higher CD4 cell count and both prevalent [23, 97] and incident hypertension [186]. One may speculate that lower CD4 cell count could be associated with normotension due to persistent immune depletion, and that lower CD4 cell count could in fact be protective of coexistent hypertension. However, other non-AIDS morbidities occur more frequently in patients with lower CD4 cell counts [22, 23, 120, 121].

It should be noted that almost all the patients with sustained hypertension and previously low nadir CD4 cell count regained a CD4 cell count level > 200 cells/ μ L at baseline (n = 29/34) (paper II). Factors related to immune reconstitution may possibly serve as potential contributors to hypertension in those with previous advanced immunodeficiency. Increased BP has been associated with lower CD4 cell count at the time of ART initiation in two prospective studies, supporting the link between immune reconstitution and increased BP observed in our study [99, 100].

In paper II, we observed that patients with low nadir CD4 cell count had higher frequency of adverse metabolic factors such as higher cholesterol and lower eGFR. Moreover, patients with previous low nadir CD4 count were more often lean with BMI < 25 kg/m². It is possible that the pathogenesis of hypertension in HIV-infected individuals may be diverse, with immunological mechanisms being more important in some, but not all patients.

In paper II, we discussed putative pathogenetic mechanisms explaining the link between low nadir CD4 cell count and hypertension; with immune activation, microbial translocation, toxic viremia, and endothelial dysfunction as possible mechanistic links. We explored the impact of viremia at the time of nadir, but found

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no link to hypertension. Unfortunately, we had no other cardiovascular or immunological data recorded at the time of nadir, and we were thus not able to investigate further the suggested mechanisms. In paper III, we showed an association between low nadir CD4 cell count and higher LPS levels. Others have also found microbial translocation increased in patients with lower CD4 cell counts [151], hence, the finding of microbial translocation as related to higher BP levels supported the connection between low nadir CD4 cell count and hypertension (paper III).

5.2.4.2 ART

In paper II, we observed that the frequency of sustained hypertension rose across groups of increased duration of ART (Fig. 7). Duration of ART was an independent predictor of sustained hypertension (paper II) and reached borderline significance as a predictor of new-onset hypertension (Paper I). ART-naïve status was negatively associated with sustained hypertension (paper II) but not with new-onset hypertension (paper I). As can be seen from Figure 7, nadir CD4 cell count < 50 cells/ μ L was most prevalent in those with prolonged ART exposure (paper II).

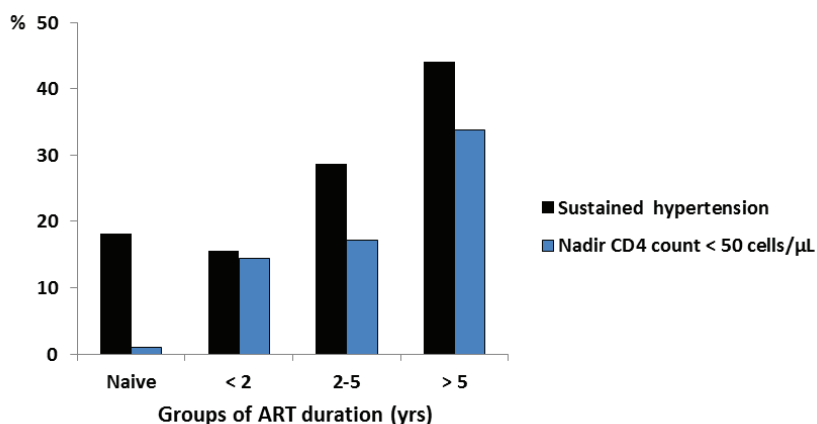


Figure 7. Distribution (%) of patients with hypertension and low nadir CD4 cell count, respectively, across groups of increased duration of ART in the longitudinal study.

DISCUSSION

As detailed earlier, several studies have demonstrated possible harmful effects of ART on BP or hypertension [77, 80, 81, 83, 90, 103], but results are conflicting [84, 86, 88, 89]. The methods of exploring impact of ART on hypertension differ in the various studies, but only a few have explored overall cumulative ART exposure [77, 81, 86, 90].

Several mechanisms may influence the observed link between cumulative ART exposure and hypertension (paper II). Patients with prolonged ART exposure might have been exposed to more toxic ART regimes with adverse impact on the vasculature. Additionally, the unfavourable effect of ART on insulin resistance, lipids and development of lipodystrophy could indirectly affect BP and partly explain the adverse influence of ART. However, ART persisted as an independent predictor of sustained hypertension when adjusting for metabolic factors in the regression models in the present study (paper II).

Many patients in our cohort reached their most severe depletion of CD4 cells around the time period when triple ART regime became available, and they might have been exposed to high-grade viremia for a longer period of time before successful ART could be initiated. Additionally, they may have experienced interrupted ART regimes more often. Interrupted ART may have led to re-emergence of viremia and to higher levels of inflammation, as demonstrated in the SMART study [187]. This important study verified that ART was in fact protective not only of HIV-related infections but also of non-AIDS events including cardiovascular disease, despite the known adverse cardiometabolic profile of ART [175, 188]. Harmful effects of interrupted ART may be applicable to hypertension as well, although this was not specifically addressed in our study.

The predictive power of ART duration seemed more pronounced in patients with low nadir CD4 cell count, which could suggest that the harmful effects of ART might be enhanced by factors related to severe immunodeficiency (paper II). Taken together, our findings may suggest that harmful effects of ART on BP may be potentiated by immune depletion and subsequent immune reconstitution. Hence,

rather than being an argument to delay ART due to side effects, our results could be interpreted in favor of commencing ART earlier, to avoid the detrimental effects of severe immunodeficiency.

5.2.4.3 *HIV duration*

In paper I, we found that duration of HIV was independently associated with increased risk of development of hypertension. We have previously reported on HIV duration as a predictor of ambulatory hypertension [101]. HIV duration reflects cumulative time spent with various harmful influences, i.e. viral replication, immune activation, low-grade inflammation, metabolic aberrations related to both HIV and ART, and mitochondrial dysfunction [189]. Consequently, these patients have been exposed to multiple stimuli which may affect endothelial function and vascular structure. In the general population, hypertension and BP elevation have been related elevation of inflammatory biomarkers such as CRP, fibrinogen and IL-6 [61-63]. These biomarkers are often elevated in individuals with HIV [189-191] and with associations to cardiovascular morbidity [192, 193], but the relationship to hypertension has not been specifically addressed.

In paper II, HIV duration did not reach statistical significance as an independent predictor of sustained hypertension. Nonetheless, the time period with HIV diagnosis and the duration of ART exposure are invariably interwoven.

5.2.4.4 *Microbial translocation*

In the exploratory substudy (paper III), levels of LPS and sCD14 were measured retrospectively at the time of nadir CD4 count. Both LPS and sCD14 levels were independently associated with increased BP level during two subsequent time periods and were associated with sustained hypertension. These findings could not be explained by cardiometabolic risk factors, viral load or CD4 cell count at the time of measurement, or by current ART or at the time of inclusion in the longitudinal study. Soluble CD14 were correlated with LPS in our study, which supported the evidence of present LPS stimulation [132, 142]. Our data suggested

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that microbial translocation and immune activation might contribute to BP elevation and even trigger hypertension.

Compared to the controls, the HIV-infected subjects in the substudy had elevated levels of triglycerides, and there was a rather close correlation between LPS levels and triglycerides [194]. Adjusting for triglycerides in multivariate analyses did not alter the finding of LPS as an independent predictor of later increased BP levels. Metabolic aberrations were not frequent in the HIV-infected patients, and elevated LPS levels caused by other influences than HIV was less probable. We found both LPS and sCD14 levels to be higher in patients with nadir CD4 cell count < 50 cells/ μ L, and the highest levels of LPS and sCD14 were in fact noted only in patients with this low nadir CD4 count. This association was in accordance with a previous Norwegian study which reported the highest sCD14 levels to occur only in patients with advanced HIV disease [195]. Furthermore, both LPS and sCD14 were correlated to nadir CD4 cell count analysed as a continuous variable in our study. The association between lower nadir CD4 cell count and LPS was in keeping with microbial translocation being linked to immune activation and progressive HIV disease [133, 144, 151, 152].

Microbial translocation and low-grade endotoxemia have not previously been associated with hypertension in the HIV-infected or HIV-uninfected population. However, low-grade elevation of LPS has been linked to metabolic syndrome of which elevated BP is one of the criteria [164, 166]. LPS may induce endothelial activation in complex with TLR4 on endothelial cells [163, 170, 196], and a connection between LPS-induced TLR4 activation of endothelial cells and coronary artery disease has been reported [197]. In a recent study, HIV-infected patients with high levels of LPS while on prolonged ART had evidence of endothelial dysfunction, suggesting an association between LPS and endothelial activation [160]. Additionally, LPS may induce activation of monocytes [198] which may stimulate development of atherosclerosis in HIV-infected patients [199]. Taken together, microbial translocation may possibly promote vascular dysfunction,

DISCUSSION

arterial stiffness and increased peripheral resistance which over time could contribute to the development of hypertension.

LPS was associated with elevated fasting insulin and triglycerides in HIV-infected individuals on ART in a Danish study [200]. Hence, microbial translocation and adverse metabolic factors could in part represent a unifying pathway, contributing to both hypertension and CVD in HIV infection.

5.2.5 Clinical implications

In the MAHO study, only 15% of the hypertensive patients had previously known hypertension. The proportion receiving antihypertensive therapy increased threefold during follow-up (paper I), indicating enhanced awareness of hypertension. Yet, a considerable proportion of the hypertensive patients remained at high risk of CVD, and many of these patients were still not receiving antihypertensive therapy at the end of the study period. Control of hypertension and proper intervention to reduce total CVD risk are important to prevent organ damage in HIV-infected populations [48, 201]. The proportion of smokers was high in our HIV-cohort. Smoking cessation should be encouraged as the HIV-infected population has excess mortality attributable to smoking compared to the general population [110, 118, 119]. Immunodeficiency may increase risk of hypertension and CVD. From that perspective, treatment of HIV infection should not be delayed until the stage of immunodeficiency. Consequently, actions to reduce the number of patients presenting with advanced HIV infection (i.e. late presenters) should be undertaken.

5.2.6 Future research

A large-scale longitudinal study is clearly warranted in order to address the impact of HIV infection on hypertension and CVD, with ample time of follow-up. The influence of low nadir CD4 count, ART exposure and immune recovery on incidence of hypertension could then be assessed. This study could also include 24-hour ambulatory BP measurements. Monitoring awareness of hypertension, control of BP and assessment of hypertensive organ damage in HIV-infected individuals over an extended time period would also be of interest.

DISCUSSION

The hypothesis of microbial translocation as a pathogenetic factor for vascular damage and hypertension should be thoroughly investigated in HIV-infected patients including use of an appropriate control group.

6 CONCLUSIONS

One third of the HIV-infected individuals in the present study were hypertensive. The incidence of new-onset hypertension was comparable to that of the general population. The prevalence of hypertension did not change during the short follow-up time, as some hypertensive individuals became normotensive. Use of antihypertensive therapy increased substantially during the short observation period, indicating increased awareness of hypertension in this patient population.

Our study demonstrated an independent relationship between HIV duration and new-onset hypertension. In addition to established risk factors, previous severe immunodeficiency, present immunocompetence, and prolonged exposure to ART were independent predictors of sustained hypertension.

We have described for the first time a possible link between microbial translocation and hypertension. Microbial translocation is a key phenomenon in HIV-related immune dysfunction and could explain the finding of low nadir CD4 cell count as a predictor of hypertension.

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8 PAPERS I–III

